Running head: AN INVESTIGATION ON ANTINOCICEPTIVE EFFECT OF *Abroma augusta* Linn. (Sterculiaceae)

By

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Certificate

This is to certify that the thesis "Antinococetive effect of *Abroma augusta*" submitted to the department of Pharmacy, East West University, Mohakhali, Dhaka, in partial fulfillment of the requirements for the degree of Bachelor of pharmacy (B.pharm.) was carried out by Gias Uddin Ahmed (ID# 2005-3-70-020) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

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Abstract

Abroma augusta Linn (Sterculiaceae), commonly known as Ulatkambal(Bengali). This plant is used as medicinal plant for its distinct pharmacological activity. Chemical analysis of this plant has noticed various chemical properties. The aim of this study was to investigate the antinociceptive effect of the ethyl acetate extract of *A.augusta* in acetic acid-induced writhing test in mice. The result suggests that the extract has exhibited significant ($p<0.05$) analgesic effects, which were comparable with standard drugs. The bark extracts at 500 and 250 mg/kg showed significant reduction in acetic acid induced writhing in mice with a maximum effect of 65.27% reduction at 500 mg/kg dose.

Key words

Abroma augusta, ulatkambal, pharmacological activity, writhing test, analgesia.

Content

Introduction

Abroma augusta Linn (Sterculiaceae) is an evergreen tree native from Asia to Australia. It grows wild throughout the hotter part of India. It also found in Java, Pakistan, Philippines and China. It will reach 10 feet (2.5 m) in height with very little spread. The leaves will reach 8 inches (20 cm) across and are 3-5 lobed with very distinct palmate veins. The leaves and stems are covered with soft bristly hairs that are very irritating to the touch. The leaves are repand, narrow and entire. The flowers are 5 cm. in diameter. The sepals are lance late. The petals are red and purple to dark red, concave, and prolonged above into a large spoon shaped lamina. The capsules are membranous. The seeds are many. The flowering season is July to August. The bark yields a jute-like fiber. The fresh viscid sap of the root bark is considered to be available emmenagogue and uterine tonic, useful in the congestive and neuralgic varieties of dysmenorrhoea.

In Ayurveda the juice of fresh leaves is very useful in female diseases of uterus and menstruation. It regulates the menstrual flow and acts as a uterine tonic. A good quality fiber is obtained from this plant. Abromine is obtained from this plant. (Waqar *et a*l, 2003)

Abroma augusta

Plant description

Taxonomy of the plant

Domain: Eukaryota

Kingdom: Plantae

Subkingdom: Viridaeplantae

Phylum: Tracheophyta

Subphylum: Euphyllophytina

Infraphylum: Radiatopses

Class: Magnoliopsida

Subclass: Dilleniidae

Superorder: Malvanae

Order: Malvales

Family: Sterculiaceae

Genus: Abroma

Specific epithet: augusta

Botanical name: - Abroma augusta

(Australian IBIS database.)

English name: Devil's Cotton, Indian hemp

Blooming *A.augusta*

Blooming Time

In the greenhouse, plants bloom from late spring to early summer. Dark maroon flowers are formed in terminal panicles. Individual flowers are up to 3 inches (7.5 cm) across.

Culture

Abroma augusta need full sun to partial shade with a rich well-drained soil mix. In the greenhouse, we use a soil mix consisting of 2 parts peat moss to 1 part loam to 2 parts sand or

perlite. Plants are watered, and then allowed to dry slightly before watering again. They are fertilized weekly during the growing season with a balanced fertilizer. They are very fast growing, so repotting should be done when they outgrow their containers. Care should be taken when repotting because of the irritating hairs. Wrapping newspaper around the trunk helps to prevent exposure to the hairs. During the winter months, water should be somewhat restricted, but not to a point where the plant loses its leaves.

Propagation

Abroma augusta is propagated from seed. Seed germinate in 21-30 days at 72° F (24°C). (Uni, Oklahoma. 2004.)

Literature review

Traditional uses

Abroma augusta Linn. is a popular medicine in the indigenous systems.In ancient time, it was being used therapeutically as an emmenagogue in diabetes insipidus with very gratifying results. Clinically it is indicated in diabetes, albuminuria, enuresis, debility, vertigo, sleeplessness, carbuncle, amenorrhoea and dysmenorrhoea. t is indicated in irregular catamenia. Infusion of fresh leaves and stems in cold water is very efficacious in gonorrhoea. Fresh viscid juice (sap) abounding in the thick easily separable bark of the root is more efficacious and given in half dranchm or 30 grains doses a days in varieties of dysmenorrhoea. A single administration during the menses will cure the disease and regulate the menstrual flow, act as a uterine tonic and bring on coinception in married women. It is generally given from the first day of the flow for 7 days successively (Panda, 2003.)

Ayurvedic treatment

The Ayurvedic treatment of sexual dysfunction in women is aimed at controlling the symptoms and treating the known cause Sexual dysfunction in women may be related to problems with desire, arousal, orgasm and pain. Medications, diseases, excessive use of

alcohol, vaginal infections, depression, relationship problems, abuse, pregnancy, breast feeding, menopause, stress, fatigue and boredom may all cause sexual dysfunction. Vaginal infections can be treated using medicines obtained from Ulatkambal (*Abroma augusta*).Cancer of the uterus, also called uterine cancer, the Ayurvedic treatment of uterine cancer is aimed at treating the endometrial tumor and preventing or reducing its spread to other parts of the body which is carried out by the same plant. (Dr. Mundewadi, 2006).

Ayurveda has good and effective treatment for dysfunctional uterine bleeding. In the chronic condition Ulatkambal is used to treat disease. This plant acts on the centers in the brain which are responsible for regulating the secretion of hormones in the blood and directly on the uterus blood vessels, which in turn act on the ovary and bring about the required therapeutic effect.). Cancer of the uterus, painful urination, painful intercourse, and pain in the pelvis area, are common symptoms of this disease. The Ayurvedic treatment is carried out by the same plant. (Dr. Mundewadi, 2006)

Antifungal activity

Antifungal activity of the oil was tested against *Trichophyton schoenleinii, Pseudalleschena boydii, Candida albicans*, *Aspergilus niger* (human pathogens), *Microsporum* canis, *Trichophyton simii* (animal pathogens). *Fusarium solani* Var. *lycopersici*, *Macrophomina phaseolina* (Plant pathogens).Growth in the medium containing the oil was determined by measuring the linear growth (mm) and growth inhibition (%) was calculated with reference to the negative control. The results (Table 1) indicated that the seed oil of *Abroma augusta* Linn. possess a moderate activity against human and animal pathogens but no significant activity of the extract was observed against the plant pathogens.The highest inhibition effects were found for *Trichophyton schoenleinii* (56%) (human pathogens) and *Microsporum canis* (50%) (animal pathogen). The oil did not exhibit appreciable activity

against the rest of fungi. This means that the seed oil of *A. augusta* Linn. Has the potential to be an atifungal agent against *Trichophyton schoenleinii* and *Microsporum* canis. (Waqar *et al*, 2003)

Phytotoxic activity

The phytotoxic activity of the oil was tested against the *Lemna aequinoctialias* Walv. Results of the phytotoxic activity of the seed oil of *Abroma augusta* Linn. on *Lemna aequinoctialias* Walv. was interpreted by analyzing the growth regulation in percentage, calculated with reference to the negative control. The results (Table 1) showed that the oil posses remarkable phytotoxic activity against *Lemna aequinoctialias* Walv. and inhibited the growth of plant by 82.35% at a concentration of 500 $\mu g\ ml^{-1}$. However, no appreciable effect was observed at a lower concentrations. This phytotoxic activity is not surprising, since some of the oil components that account for 1% or greater of the oil, such as eugenol lodil and viridiflorol are known to be phytotoxic. (Waqar *et al*, 2003)

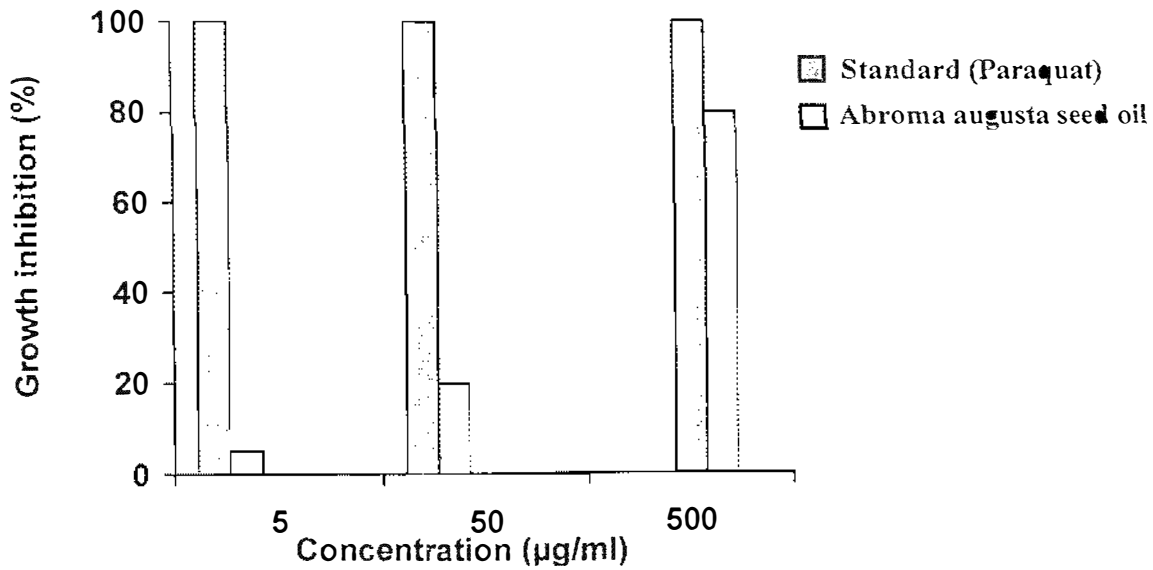


Fig. 1: Phytotoxic activity of *Abroma augusta* Linn seed oil. Here seed oil was compared with standard(paraquat) to evaluate the percent growth inhibition.

Table 1: Susceptibilities of different fungi to *Abroma augusta* Linn.seed oil

	Linear Growth (mm)		Inhibition	
Name of fungi	**Sample**	**Control**	**(%)**	**Standard Drugs**
Human pathogens				
Trichophyton schoenleinii	20±1.8	45±2.1	55.55	Miconazole
				Ketoconazole
Pseudalleschena boydii	30±2.3	55±3.0	45.54	Miconazole
				Ketoconazole
Candida albicans	50±3.1	70±3.7	28.57	Miconazole
				Ketoconazole
Aspergilus niger	37±2.6	60±3.8	38.33	Amphotericine
Animal pathogens				
Microsporum canis	25±3.6	50±4.1	50	Miconazole
				Ketoconazole
Trichophyton simii	40±2.1	60±3.8	33.33	Miconazole
				Ketoconazole
Plant pathogens				
*Fusarium solani*Var.	30±1.6	50±4.1	40	Benlate
Macrophomina phaseolina	60±5.6	60±4.3	0	Benlate

Antifungal activity of *Abroma augusta* Linn seed oil (Waqar *et al*, 2003)

Antibacterial activity

The oil was screened against various human pathogens including *Corynebacterium diphtheriae, Escherichia coli, Klebsiella pneumoniae, Proteus morganni, Pseudomonas aeruginosa, Salmonella typhi, Shigella Boydii, Staphylococcus aureus and Streptococcus pyogenes* by agar well diffusion method .The oil did not display remarkable results for antibacterial activity. (Waqar *et al*, 2003)

Anti diabetic activity

The combined effect of water extract of a mixture *Abroma augusta* roots and *Coccinai indica* leaves in streptozotocin induced diabetic in rats is reported.The results obtained with untreated diabetic rats and diabetic rats treated with *A. augusta* plus *C. indica* on fasting blood glucose were compared with normal healthy controls. It is seen that treatment with water extract of *A. augusta* plus *C.indica* at a dose of 300mg / kg body weight brought down fasting blood glucose from a higher value of 166.9 ±25.4 mg/dl to a normal value of 85.4±2.3 mg/dl while in the untreated group the fasting blood glucose increased from the initial value of 172.2 ±5.4 to 285.6±42.6 mg/dl. There was considerable fall in fasting blood glucose in diabetic rats treated with *A. augusta* alone. But the effect of the two plants in combination was more than that with either of the plants alone. Similar improvement to normal glucose tolerance was seen in Glucose tolerance test. In the diabetic untreated rats the blood sugar was 269.0 ± 92.2 mg/dl even after 2 hrs of glucose load in glucose tolerance test. But in the *A. augusta* plus *C. indica* treated rats the 2 hr blood glucose value was in the normal range of 75.2±1.0 mg/dl. The fasting (0hr) blood glucose values which were higher in the diabetic animals (160.5 ± 32.1) were brought down to 81.0 ± 3.5mg/dl, when 300 mg of the extract of the mixture of the two plants was administered for 8 weeks. However it is possible that these extracts increase blood insulin levels and also stimulate utilization of glucose by liver and extrahepatic tissues. (.Halim *et al* 2003)

Hypoglycemic effect

For the study on the hypoglycemic effect of *A.augusta* in Alloxan diabetic rats the dried whole root bark powder was extracted by distilled water. For the experiment animals were divided into 3 groups of 5 animals each. Group I served as healthy controls and group 2 were untreated diabetic rats. Animals of both the group were given water for 16 weeks. Group 3 were diabetic rats given 4 ml (4gm dry wt.) of aqueous extract of *A.augusta* daily

once using an intragastric tube for 16 weeks. Plasma glucose levels, fasting as well as in oral glucose tolerance test and serum lipid profile were determined. This study reports not only the anti-hyperglycemic effect but also for the hypolipidemic effect of *A.augusta.*

Results in table I show the effect of water extract of *A. augusta* on the fasting plasma glucose of normal and untreated diabetic rats. It can be seen that treatment rats with 4 ml of water extract of *A. augusta* brought down the fasting plasma glucose level to normal value. Treatment of diabetic rats with 4 ml of extract showed considerable improvement in glucose tolerance (Table 2.2). These results point out that the diabetic rats showed abnormal glucose pattern. Alter treatment for 16 weeks with *A. augusta* the glucose tolerance pattern was normal.

Table 2.1: Effect of water extract of *A. augusta* on the fasting plasma glucose level in rats

Group	**Plasma glucose mg/dl, mean + S.D**	
	0 weeks	**16 weeks**
Normal	95.5±26.6	84.0±29.3
Diabetic untreated	166.7±50.4	300.7±50.9
Diabetic + *Abroma* (4 ml) treated	164.6±26.9	107.5±28.6

Table 2.2: Effect of 4 ml of *A. augusta* extract on glucose tolerance in diabetic rats after16 weeks treatment.

Group	**Plasma glucose mg/dl, mean ± S.D**				
	0 hr	**0.5hr**	**1hr**	**1.5 hr**	**2 hr**
Normal	96.5±26.9	142.6±14.8	135.2±30.2	118.5±11.4	106.0±14.0
Diabetic	155.7±42.0	240.6±78.1	271.0±91.1	284.7±81.8.	275.0±85.2
Diabetic treated	89.8±25.2	115.0±21.1	116.4±16.0	104.0±11.4	99.8±25.0

Table 2.3: Effect of treatment with water extract of *A. augusta* on the serum lipid profile in diabetic rats.

Group	Serum lipids				
	TC (mg/dl)	LDLC (mg/dl)	HDLC (mg/dl)	LDLC/ HDLC	TAG (mg/dl)
Normal	168.6±16.9	78.0±12.2	44.9±13.3	1.7±0.3	115.6±42.6
Diabetic untreated	246.0±14.4	152.2±12.6	45.0±12.0	3.3±0.4	181.8±18.8
Diabetic treated	186.0±14.8	99.7±16.8	51.3±8.4	1.9±0.4	175.0±15.8

TC, LDLC, HDLC= total, low density and high density lipoprotein cholesterol respectively. TAG= triacylglycerol.

In the diabetic rats there was increase in total-and LDL- cholesterol, LDL/HDL ratio and tdacylglycerois (Table 2.3). After treatment with 4ml. of *A. augusta* extract, there was significant lowering of the serum lipids. There was slight increase in HDL- cholesterol. This indicates that the water extract of *A. augusta* has effect on the lipid metabolism of diabetic rats. It is anticipated that isolation of the active hypoglycemic compound(s) and detailed work on its effect on carbohydrate and lipid metabolism and blood insulin levels would throw light on mechanism of hypoglycemic action of *A. augusta*. (Halim *et al* 2001)

Chemical Investigation of *Abroma augusta* Linn

Chemical composition

Little or no previous work has been done on this drug. To test for the presence of alkaloids of roots, the powdered root was extracted with prollius liquid. The extract taken up in dilute HCI gave all the reactions for alkaloids. The amount, however, was less than 0.01%, the petroleum ether extract showed the presence of a fixed oil and a little resinous matter; the ethereal solution gave further amount of resin, the alcoholic extract showed the presence of

doubt its identity with betaine in view of our isolation of the letter from the roots. The non volatile, non saponifiable fraction of the petroleum ether extract of the roots on chromatography over aluminum oxide yielded 2 sterols giving a violate to green color (Through blue) with Liebermann-Burchard reagent. These were identified as β-sitosterol (m/e414 m^+) and stigmasterol(m/c 412 m^+) .

The petroleum ether extract of the leaves of *Abroma augusta* on similar treatment yielded the following 4 compounds having different Rf values as revealed by thin-layer chromatography (SiO_2; C_6H_6, $CHCl_3$, 1:1; I_2 vapor or AC_2O-H_2SO_4-EtOH mixture as developer)

1. Compound A from petroleum ether eluant, granular solid, mp 84-85° (EtOAC), Rf 0.35 (I_2 vapour) identical spot with octacosanol, freely soluble in pet. Ether, C_6H_6. and gave no coloration with Liebermann-Burchard reagent. IR-absorpson peaks at 3220(OH),2860,1465,1400,1380,1125,1075,1065, 1020,735and722[-$(CH_2)_n$ -rocking split] cm^{-1} in Nujol, which compared favourably well with those of octacosanol. NMR-spectrum in $CHCl_3$ showed a prominent peak at 1.25δ due to methylene protons and small peaks at 0.9δ and 3.65 δ. The mass spectrum exhibited prominent higher mass peaks at m/e 392 (M-18) and (M -28) with a smaller mass peak at m/e 450,451,436,423,408,407,394,393,378, etc. The general fragmentation pattern of the mass spectrum indicated the compound to be a mixture of octacosanol.
2. Compound B from petroleum ether eluant, fine needles, mp 260-268 ° (pet. Ether),Rf value 0.95, soluble in pet.ether, C_6H_6, $CHCl_3$, gave a pink color with Liebermann-burchard reagent. IR- spectrum was very similar to that of compound C with additional absorptions at 3250 cm^{-1} (Primary OH) and 722and 735 cm^{-1} [-$(CH_2)_n$ - rocking split]. NMR-spectrum in $CHCl_3$ showed a prominent methylene proton peak

at 1.25δ and methyl proton peaks at 0.82,0.95,1.1 and 1.6 δ .This comparative study indicate a compound to be a mixture of taraxerole.

3. Compound C from pet. ether: C_6H_6 (1:1) eluent, crystalline rods, mp 279-280 ^{o} (C_6H_6), Rf value 0.91.Sparingly soluble in acetone C_6H_6, $CHCl_3$, insoluble in pet. ether, soluble in acetone, ethyl acetate and alcohol and gave a pink color Liebermann-Burchard reagent. The mass spectrum exhibited the usual fragmentation pattern of taraxerol.
4. Compound D from C_6H_6 and $CHCl_3$ eluents, needles, mp 134-135^{o} (alcohol), Rf value 0.28, gave a violet to blue to green color with Liebermann-burchard reagent. (MITSUYASU,1969)

Chemical analysis for seeds

The seeds of *Abroma augusta* (2kg) were pulverized into powder and extracted with n-hexane in Soxhlet apparatus. The solvent was evaporated at low temperature under reduced pressure in rotary evaporator to obtain the oil (100g).

The oil (1g) was treated with boiling methanol (50ml) in the presence of concentrated hydrochloric acid (5ml) and was refluxed (5h) at 150 C. After cooling to room temperature, the methyl esters thus formed were extracted with ethyl acetate (30ml X 3). The ethyl acetate was evaporated under reduced pressure in a rotary evaporator to obtain the esterified oil. (Waqar et al, 2003)

The fatty acids composition of the esterified oil was determined by gas chromatography and mass spectrometer. The analysis was performed using gas chromatograph coupled with mass spectrometer (JMS-HX 110,Jeol).The Capillary column (OV-101) with an internal diameter of 0.25mm was used. The temperature was raised from 70 to 260 o C. Helium was used as the carrier gas at a flow rate of 2 ml min^{-1} split ratiol 80^{-1}. The significant ions in the mass spectra of these acids are as follows:

- n-Hexadecanoate, methyl ester: 270(M^+ ,$C_{17}H_{34}O_2$)
- 2-methyl hexadecanoate, methyl ester: 284(M^+ ,$C_{18}H_{38}O_2$)
- n-Octadecanoate, methyl ester: 298(M^+ ,$C_{19}H_{38}O_2$)
- 2-methyl octadecenoate, methyl ester: 312(M^+,$C_{20}H_{40}O_2$)
- 9-Octadecanoate, methyl ester: 296(M^+ ,$C_{19}H_{36}O_2$)
- 10-nonadecenoate, methyl ester: 310(M^+ ,$C_{20}H_{38}O_2$).(Waqar *et al*, 2003)

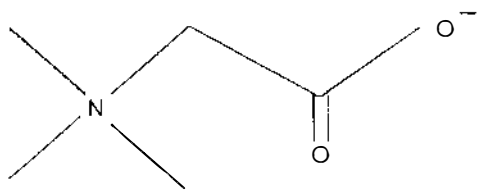
Table 5 shows the relative percentages of fatty acids in the total seed oil of *Abroma augusta* Linn. A total of six fatty acid methyl ester were identified, four of them being the saturated (66.6%) and two unsaturated (33.3%), in the seed oil.

Table 3: Fatty acids identified in the seed oils of *A.augusta* Linn

Fatty acids	MOL. WT.	% of Whole oil
Saturated fatty acids		
n-Hexadecanoate, methyl ester		
CH_3-$(CH_2)_{14}$-CO_2CH_3	270	33
2-methyl hexadecanoate, methyl ester		
CH_3-$(CH_2)_{13}$-CH-CO_2CH_3	284	28
n-Octadecanoate, methyl ester		
CH_3-$(CH_2)_{15}$-CO_2CH_3	298	13
2-methyl octadecenoate, methyl ester		
CH_3-$(CH_2)_{15}$-CH-CO_2CH_3	312	01
Unsaturated fatty acids		
9-Octadecanoate, methyl ester		
CH_3-$(CH_2)_7$-CH=CH$(CH_2)_7$ -CO_2CH_3	296	23
10-nonadecenoate, methyl ester		
CH_3-$(CH_2)_7$-CH=CH $(CH_2)_8$ -CO_2CH_3	310	02

(Waqar *et al*, 2003)

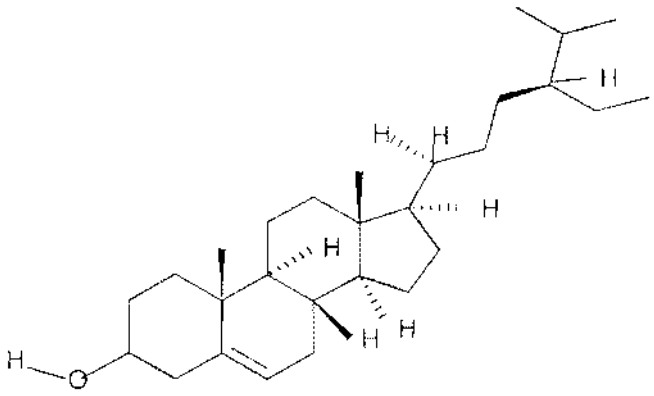
Chemistry of Active Ingredients



Structure: Abromine

IUPAC Name: Crboxymethyl-trimethyl-ammonium

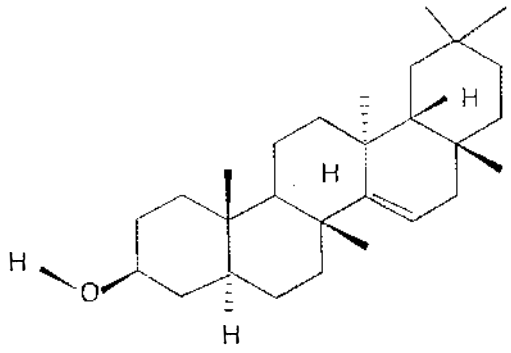
Formula: $C_5H_{12}NO_2$



Structure: Taraxerol

IUPAC Name: 4,4,6a,6a,8a,11,11,14b-octamethyl-1,2,3,4a,5,6,8,9,10,12,12a,13,14,14a-tetradecahydropicen-3-ol

Formula: $C_{30}H_{50}O$



Structure: β-Sitosterol

IUPAC Name: 17-(5-ethyl-6-methyl-heptan-2-yl)-10, 13-dimethyl-2,3,4,7,8,9

Formula: $C_{29}H_{50}O$. (Dr. A. A. Mundewadi,2006)

Materials and methods for writhing test

Plant material

The fresh whole plant was *Abroma augusta* (family: Sterculiaceae) was collected from various places of Sirajgonj district. It was identified by the Bangladesh National Herbarium, Mirpur, Dhaka as *Abroma augusta*. Accession number DACB-34196

The bark was collected in fresh condition. It was shade dried effectively to make it suitable for grinding purpose. The powder was then stored in air tight container in cool, dark and dry place for proper use.

Preparation of extract

The dried and plant material (50g) was macerated in ethyl acetate (200ml) in an air tight flat bottom container for 72 h with occasional shaking and stirring. The ethyl acetate extract was then filtered and evaporated under pressure using rotary evaporator. A rotary evaporator is a device was used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation.

Drugs

Diclophenac sodium BP was provided by Square pharmaceuticals Ltd. (Bangladesh), acetic acid Merk, Germany.

Experimental animal

The experimental animals (Swiss albino mice) were obtained from the Animal Research Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR,B) weighing18–26 g, housed in polyvinyl cages on light–dark cycle at 22±2°C with access to food and water *ad libitum*. To keep the hydration rate constant, food and water were stopped 12 hours before the experiments. Experiments were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation

of experimental pain, approved by the Animal Care and Use Committee of the Third Faculty of Medicine, Charles University.(Zimmermann, 1983). Animals were (1) acclimatized to the laboratory for at least 1 h before testing,(2) were used only once during the protocol, and (3) were sacrificed, by an anesthetic overdose, immediately after anlgesiometric testing. The duration of the experiments was as short as possible and the number of animals was the minimum compatible with consistent effects of drug treatments (six mice per experimental group). All procedures involving animals strictly adhered to the guidelines proposed by the Committee on Research and Ethical Issues of the International Association for the Study of Pain for investigations of experimental pain in animals (Zimmermann, 1983).

Apparatus and Reagents

Mouse box (10), mice feeding needle, 100 unit syringes (2ml), ethyl acetate (2L), acetic acid (20mL), tween-80(2ml).

Preparation of the Test Material and the Standard

In order to administer the crude extract of *A.augusta* (ethyl acetate) at a dose of 250mg/kg and 500mg/kg body weight of mice, of the extract were measured and were triturated unidirectional by the addition of small amount of Tween-80. After proper mixing of the extract and Tween-80, normal saline was slowly added. The final volume of the suspension was made 10ml. To stabilize the suspension it was stirred well by using vortex mixer.

The inject able preparation of the standard (diclophenac) was used at the dose of 50 mg/kg body weight. The peripheral analgesic activity of the ethyl acetate extracts of *A.augusta* bark was determined by the acetic acid induced writhing method. The inhibition of writhing in mice by the plant extract was compared against inhibition of writhing by a standard analgesic agent (diclophenac), given intraperitoneal at a dose of 50 mg/kg body weight.

The writhing test

The writhing test was selected as a model of acute visceral pain because it is a feasibly reproducible, widely accepted, and well-established pain test used in laboratories around the world. It is the abdominal constriction test is used to measure the antinociceptive actions of analgesics A writhe was defined as a wave of contraction of abdominal muscles followed by dorsiflexion and extension of the hind limbs.The peripheral analgesic activity of bark extract of *Abroma augusta* was measured by the acetic acid induced writhing test. The inhibition of writhing produced by plant extract was determined by comparing with the inhibition produced by the control group. (Millan 1994; Miranda et al, 2001)

Procedure

24 experimental animals (mice) group were selected and divided into 4 groups, denoted as:

Group 1: Negative control

Group 2: Positive control

Group 3: Sample, 250mg/kg

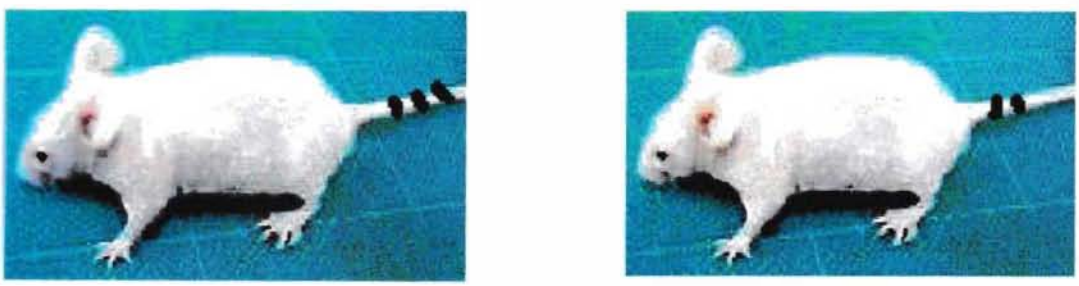
Group 4: Sample, 500mg/kg,

Each group of mice was marked individually in tail with marker to differentiate from each other. This helped to monitor all mice properly and to pick up data accurately.

Mice were injected intraperitoneally with a 0.1ml/10g of 0.6% acetic acid solution used to create pain sensation, 30 min after administration of the control and test sample. The 30 min interval was established during preliminary experiments as the optimal interval for achieving the maximal effect. Mice were injected with acetic acid in group, which were then placed in a clear polyvinyl cage (20×30×20 cm) for observation. The number of writhes in a 30 min period was counted, starting 5 min after administration of the acetic acid. Antinociception was expressed as percent inhibition in the number of writhes observed in

sterile saline water control animals during the 30-min period. Each group of six animals was observed by six observers who were blinded to the treatment. Thirty minutes before the start of the writhing test, animals were intraperitoneally administered with (1) the negative control (sterile saline water), (2) the positive control (diclophenac, 50.0 mg/kg), and orally administered with (3) the extract 250mg/kg and (4) the extract 500 mg/kg to asses the antinociceptive effect via isobolographic analysis.

Each mouse was weighted properly and the mice were marked with suitable marker in the tail for proper monitoring. The dose of the test sample and control materials were adjust accordingly. Test sample is given orally by means of long mice feeding needle with ball shaped end to the mice. The number of writhing was calculated for 30min, 5min after the application of acetic acid. (Fig 2).



Swiss albino mice (marking in tail)

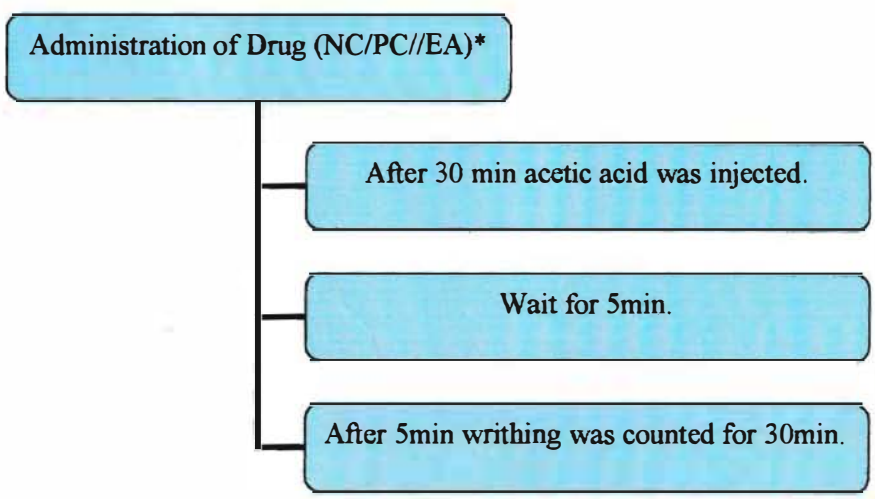


Fig. 2: Writhing count protocol.

* Here,NC=

EA= Ethyl acetate extract

Data analysis

Table 4.1: Negative control

No of mice	Body weight of mice (gm)	Writhing no.	Average writhing
1	21.1	19	
2	19.5	20	
3	25.9	17	
4	24.5	19	20.16
5	19.3	28	
6	20.6	18	

Table 4.2: Positive

No of mice	Body weight of mice (gm)	Writhing no.	Average writhing
1	18.9	1	
2	19.8	6	
3	18.0	0	
4	23.4	1	2.16
5	25.0	3	
6	24.6	2	

Statistical analysis

The data was analyzed by one way ANOVA using SPSS-10 for Windows, subjected to Dunnett t testin post hoc and expressed as statistical mean ± standard error of mean differences between means werte regarded significant at$P<0.05$.

Table 4.3: Extract 250mg/kg

No of mice	Body weight of mice (gm)	Writhing no.	Average writhing
1	20.2	8	
2	21.4	2	
3	22.8	13	
4	19.0	11	10.16
5	24.0	8	
6	20.0	19	

Table 4.4: Extract 500mg/kg

No of mice	Body weight of mice (gm)	Writhing no.	Average writhing
1	18.4	7	
2	19.5	3	
3	25.4	10	7
4	14.7	4	
5	19.5	6	
6	21.6	12	

Results

The antinociceptive effect of ethyl acetate of *A.augusta* is shown in table 5. The extract at doses of 250 and 500 mg/kg

by acetic acid which were compared with the standard (diclophenac).The 250 mg/kg extract causes 49.6% inhibition of writhing and the 500 mg/kg extract causes 65.27% inhibition of writhing compared to negative control.

Table 5 :Data analysis with one way ANOVA.

ANOVA

Writhing inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between groups	992.458	3	330.819	14.852	000
Within groups	445.500	20	22.275		
Total	1437.958	23			

Post Hoc Tests

Multiple Comparisons

Dependent variable:
Writhing inhibition.

Group (I)	Group (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Bonferroni						
1	2	-17.33	2.72	.000	-25.31	-9.36
	3	-11.67	2.72	.002	-19.64	-3.69
	4	-13.17	2.72	.001	-21.14	-5.19
2	1	17.33	2.72	.000	9.36	25.31
	3	5.67	2.72	.304	-2.31	13.64
	4	4.17	2.72	.851	-3.81	12.14
3	1	11.67	2.72	.002	3.69	19.64
	2	-5.67	2.72	.304	-13.64	2.31
	4	-1.50	2.72	1.000	-9.48	6.48
4	1	13.17	2.72	.001	5.19	21.14
	2	-4.17	2.72	.851	-12.14	3.81
	3	1.50	2.72	1.000	-6.48	9.48
Dunnett T3						
1	2	-17.33	2.72	.004	-26.94	-7.73
	3	-11.67	2.72	.004	-18.05	-5.28
	4	-13.17	2.72	.015	-22.97	-3.37
2	1	17.33	2.72	.004	7.73	26.94
	3	5.67	2.72	.363	-3.94	15.27
	4	4.17	2.72	.788	-7.31	15.34
3	1	11.67	2.72	.004	5.28	18.05
	2	-5.67	2.72	.363	-15.27	3.94
	4	-1.50	2.72	.995	-11.24	8.24
4	1	13.17	2.72	.015	3.37	22.97
	2	-4.17	2.72	.788	-15.34	7.01
	3	1.50	2.72	.995	-8.24	11.24

The inhibition of the nociceptive response considering significance ($P<0.05$) value that compared with each group. The value of each group is neither greater nor equal to P value 0.05, that indicate significant response.

Table 6: percentage inhibition of writhing for each group

Treatment	**Mean writhing**	**% inhibition**
Negative control	20.16	0
Positive control	2.16	89.28
Extract 250mg/kg	10.16	49.6
Extract 500mg/kg	7	65.27

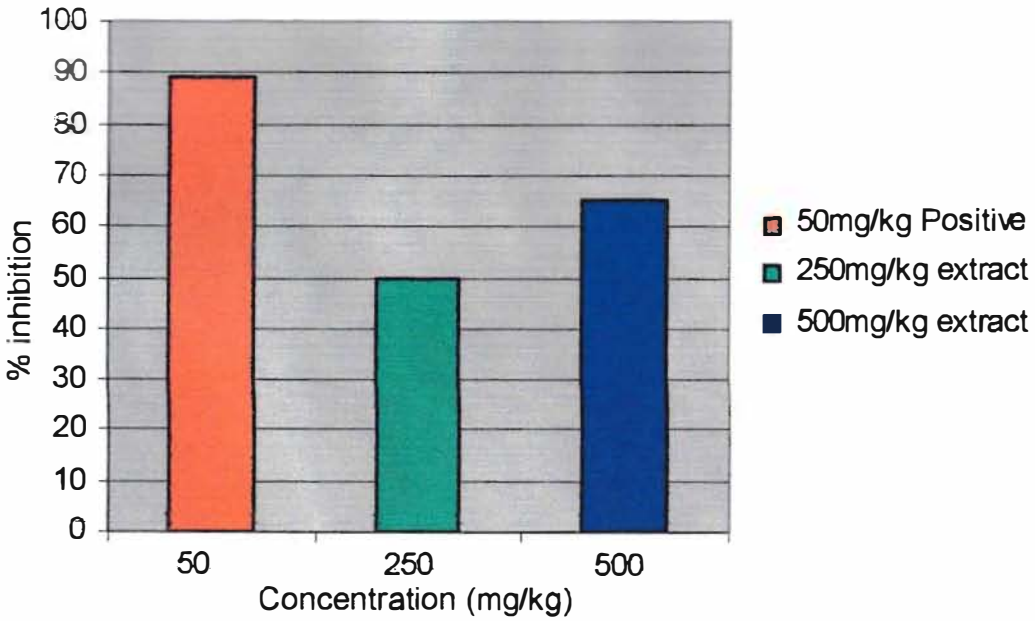


Fig. 3: Percent inhibition with concentration of each group

Discussion

Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptors or irritation of the visceral surface, which lead to liberation of histamine, bradykinins, prostaglandins, serotonin and their levels were increased in the

peritoneal fluid of the acetic acid induced mice. The constriction response of abdomen produced by acetic acid is a sensitive procedure for peripheral analgesic agents. This response is believed to be mediated by the prostaglandin pathways (Deraedt et al., 1980).

The extract of *A. augusta* produced antinociceptive activity and thus indicated the presence of analgesic components that might influence the prostaglandin pathways. Therefore, the inhibition of writhing by the extract of *A. augusta* could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. We know if p value between any two groups is greater or equal to 0.05, then the response of the two groups are equal. The figure 1 shows significant difference in p value among the four groups naming negative control (G-1), positive control (G-2), extract 250mg/kg (G-3), extract 500mg/kg (G-4).So *Abroma augusta* (250 and 500mg/kg) produced a significant ($p<0.05$) dose-related inhibition of acetic acid-induced abdominal writhing by 49.6 and 65.27%, respectively,(table 10) when compared to the control group. Maximum writhing has found for group-1 and less writhing has found for group-2.The group-3 and group-4 are in between them. This implies that writhing inhibition of plant extracts are significant.The mechanism of analgesic effect of extracts of *A. augusta* bark could probably due to blockage of effect or release of endogenous substances that excite pain nerve endings.

Overall we can say that ethyl acetate extract of *A. augusta* bark showed antinociceptive activity in acetic acid induced writhing test.

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